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# **Co-application of activated carbon and compost to contaminated soils: Toxic elements mobility and PAH degradation and availability**

**Short title:** Co-application of activated carbon and compost to contaminated soils

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## **Co-application of activated carbon and compost to contaminated soils: Toxic elements mobility and PAH degradation and availability**

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### **Abstract**

This work assesses the suitability of three commercial activated carbons (AC) and their combination with olive mill waste compost (AC+C) as amendments for the remediation of two different contaminated soils. The treatments were applied to a mining soil and their ability to immobilize trace elements was evaluated. Besides, the efficiency of the amendments to degrade and reduce the availability of polycyclic aromatic hydrocarbons (PAH) was investigated in a soil from a wood creosote treatment plant. To this aim, trace elements mobility and PAH degradation and availability were evaluated. Ecotoxicological assays were performed to assess potential toxicity risks in the untreated and the amended soils. In the mining soil, the AC were able to immobilize metals and As but the AC+C treatments were more effective than AC. In the PAH-polluted soil, AC treatments promoted the degradation of high molecular weight PAH but the AC+C amendments further enhanced the degradation of total PAH and reduced the availability of those with 3-rings. The ecotoxicological tests demonstrated an improvement of soil quality when AC and compost were applied together. In conclusion, the co-application of AC and compost reduces the mobility of potentially toxic elements in the polluted mine soil and enhances PAH degradation and reduces PAH availability in the creosote-contaminated soil.

**Keywords:** soil remediation, adsorption, metals, arsenic, organic pollutants, polycyclic aromatic hydrocarbons

### **1 Introduction**

Remediation of soils frequently focuses on the reduction of pollutants toxicity and the recovery of the biogeochemical cycles of the soil. In this sense, the use of amendments is a widely used strategy to improve the soil physico-chemical properties and nutrients availability (Alburquerque et al. 2011; Pardo et al. 2014; Clemente et al. 2015), stimulate soil microbial population and activity (Pardo et al. 2014; García-Delgado et al. 2015a, b) and reduce pollutants concentration and/or bioavailability (Beesley et al. 2010, 2014; Karami et al. 2011; Alburquerque et al. 2011; Pérez-Esteban et al. 2014; Manzano et al. 2014). Nevertheless, although the reduction in pollutants mobility is a main objective of a remediation

process, the ultimate goal should be revegetation of the affected area and the recovery of soil functions (Arienzo et al. 2004; Pardo et al. 2014).

The application of organic amendments is very interesting due to their high potential to enhance the degradation of organic pollutants and the immobilization of inorganic and organic pollutants (Beesley et al. 2011; Wu et al. 2013; Clemente et al. 2015). However, the mechanisms of soil remediation and its effectivity depend on the physico-chemical characteristics of the soil and pollutants and the stability of organic matter (OM) applied (Sayara et al. 2010; Pérez-Esteban et al. 2014).

Carbonaceous materials, such as biochar or activated carbon, show high capacity to adsorb pollutants as a result of their chemical structures, high porosity and large surface area (Denyes et al. 2013). Therefore, biochar and activated carbon tend to immobilize organic and inorganic contaminants and minimize their dissolution and bioavailability (Zhang et al. 2013; Denyes et al. 2013; Brennan et al. 2014), reducing pollutants leaching into groundwater and their transfer to the food chain (Hale et al. 2012; Jakob et al. 2012). The adsorption mechanisms of metals on activated carbons and biochars include: ionic exchange, co-precipitation with mineral oxides, complexation with functional groups such as hydroxyl or carboxyl and physical adsorption (Zhang et al. 2013). The key factors which govern the adsorption of organic pollutants on chars are the high surface area, hydrophobicity (low O+N content), aromatic nature and micro- and mesopore volume (Li et al. 2002; Zhang et al. 2013). The adsorption of organic pollutants onto these materials could reduce their degradation rate by soil microorganisms by decreasing pollutants bioavailability (Beesley et al. 2011; Zhang et al. 2013), but on the other hand, the addition of these soil amendments can reduce the toxicity of the pollutants, thereby increasing the microbial degradation rate of organic contaminants (Beesley et al. 2010; Gomez-Eyles et al. 2011; Marchal et al. 2013a; Anyika et al. 2015).

Another common organic amendment used for soil remediation is compost, which is produced by microbiological oxidation of organic residues. Compost contains a high proportion of humidified OM, which can decrease the bioavailability of metals in soil by adsorption and formation of stable complexes with humic substances (Clemente et al. 2015). Also, compost reduces the bioavailability of organic pollutants, such as polycyclic aromatic hydrocarbons (PAH) (Wu et al. 2013). However, the decrease of PAH bioavailability does not always mean low PAH degradation. In diesel spiked soil, compost addition initially decreased PAH degradation by up to 89% because of the decrease in bioavailability resulting

from strong sorption irrespective of compost type; but as the experiment advanced, compost amendment enhanced PAH degradation by more than 2-fold compared with the unamended soil (Wu et al. 2013). An important factor is the maturity grade of the organic matter applied; stable compost can accelerate PAH degradation because humic matter facilitates the desorption of the PAH, increasing their availability for microorganisms (Sayara et al. 2010). In addition, the application of compost involves nutrients and organic soluble carbon input in soil, which results in biostimulation of soil microorganisms and enhances organic pollutants degradation (Tyagi et al. 2011; García-Delgado et al. 2015c, b; Lukic et al. 2016). Despite all these benefits, some composts can lead to metal mobilization through formation of soluble complexes with dissolved organic carbon (DOC) (Herrero-Hernández et al. 2011; Pérez-Esteban et al. 2014).

Consequently, in view of the benefits and limitations of both organic amendments, activated carbon and compost, to remediate polluted soils when applied individually, their combination should be investigated as a novel approach to enhance the positive effects of both amendments on soil remediation processes (Beesley et al. 2014; Karer et al. 2015; Rodríguez-Vila et al. 2015). The aim of this work is to determine the effects of the single application of three commercial activated carbons or in combination with compost in two polluted soils. One soil was collected from an abandoned mine and is contaminated by heavy metals and As (inorganic pollutants). The other one is from a creosote wood treatment plant and is polluted by PAH (organic pollutants). This work evaluates: i) the mobility of potentially toxic elements in the mine soil, ii) degradation and bioavailability of PAH in creosote polluted soil and iii) residual soil ecotoxicity upon treatments addition.

## **2. Materials and methods**

### **2.1 Materials**

A soil polluted with potentially toxic elements was collected from abandoned tailing dumps in a silver mine located at Bustarviejo (Madrid, Spain). A detailed description of the area can be found in previous works (Moreno-Jiménez et al. 2009, 2010). PAH polluted soil was taken from a creosote wood treatment plant (Castejón, Spain) where sleepers were impregnated. Creosote is a mixture chemical formulation with 85% of PAH, compounds with proven carcinogenic, mutagenic and toxic characteristics (IARC 2010), so the remediation of these soils prevents environmental damage and human diseases. Both soils were homogenized, air-dried and sieved to <2 mm. Olive mill waste compost (C) was prepared by mixing

olive mill waste and cow manure (11% on a dry weight basis), with a total composting time of 36 weeks (Albuquerque et al. 2011). The main characteristics of the soils and the compost are shown in Table 1. Three different commercial activated carbons were used: Chemviron Centaur HSL 8x30 (AC1), Merck K30263614 (AC2) and Norit Row 0.8 Supra (AC3). The characteristics and porous structure of the AC (Table 2) were determined according to the methodologies described in Calvo et al. (2006) and Rey et al. (2011).

## 2.2 Experiment design

Polluted soils (60 g) were amended with three AC individually and in combination with compost at doses of 1% ( $25 \text{ t}_{\text{AC}} \text{ ha}^{-1}$ ) and 2% ( $50 \text{ t}_{\text{C}} \text{ ha}^{-1}$ ) (w:w) respectively. The dose of AC (1%) was selected based on the results of Kołtowski et al. (2016) and Karer et al. (2015), who demonstrated the effectivity of low doses of AC to immobilize PAH and the capacity of biochar at 1.5% and mixed with compost to immobilize metals in soil, respectively. The mixtures were placed in 50 mL plastic centrifuge tubes for the mining soil and in 100 mL glass Erlenmeyer flasks for creosote polluted soil. The resulting treatments were:

- 1: Unamended soil (S)
- 2: Soil + AC Chemviron at 1% (AC1)
- 3: Soil + AC Merck at 1% (AC2)
- 4: Soil + AC Norit at 1% (AC3)
- 5: Soil + AC Chemviron at 1% + Compost at 2% (AC1+C)
- 6: Soil + AC Merck at 1% + Compost at 2% (AC2+C)
- 7: Soil + AC Norit at 1% + Compost at 2% (AC3+C)

All treatments were carried out per quadruplicate. The microcosms were maintained at 60–70% of their water holding capacity (WHC) by weighing and adding water losses. The experiment was carried out in darkness at room temperature for 35 days.

## 2.3 Pore water sampling and analysis

Metal(loid)s mobility was evaluated in the mining soil pore water. One Rhizon sampler (Eijkelkamp Agrisearch Equipment, The Netherlands) was inserted in each reactor and soil pore water was extracted after 35 days of incubation. Approximately 5 mL of each pore water sample were taken for pH and EC analyses prior to elemental analysis. Dissolved organic carbon (DOC) was determined using a TOC-V analyzer (Shimadzu). Pore water samples were conserved in 1% HNO<sub>3</sub> for heavy metals and As concentration analysis by ICP-OES (Iris Intrepid II XDL, Thermo Scientific). For the analysis of As speciation, pore water aliquots were diluted with 5 mM Na<sub>2</sub>-EDTA, stored at -20 °C and As speciation was determined according to Fresno et al. (2016).

#### 2.4 Total and bioavailable extraction and analysis of PAH.

The total extraction of PAH with acetone: hexane (1:1) and further analysis by HPLC-PDA were performed according to García-Delgado et al. (2013). The PAH bioavailable fraction for soil microorganisms was extracted with hydroxypropyl-β-cyclodextrin (HPCD) (Stokes et al. 2005). The PAH detected and quantified were: acenaphthene (Ace); fluorene (Flu); phenanthrene, (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBahA), benzo[g,h,i]perylene (BghiP) and indeno[1,2,3-c,d]pyrene (IcdP).

#### 2.5 Ecotoxicology tests

The luminescence inhibition of *Vibrio fischeri* was performed according to the standardized method ISO 11348-3 (ISO 2007) using a Biotox Kit (Aboatox). Soil leachates were diluted with NaCl 2 % (w/v) to obtain concentrations of 100, 50, 25, 12.5, 6.25 and 0% (v/v). The decrease of light emission was tested 30 min after a suspension of the bacteria was put in contact with the sample using a luminometer Optocom I (MGM Instruments). The results are thus expressed as the % of the soil leachates that reduced the bacteria luminescence by 50% in the contact time period (EC<sub>50</sub>).

A seed germination test using *Lactuca sativa* L. was performed in petri dishes containing 20 g of each soil (at 80% WHC). Ten seeds of *L. sativa* were placed directly on soil (moisture 80% WHC) and were incubated at 28 °C in darkness for 96 h. The successfully emerged plants (those with root elongation) were counted and the emergence success was calculated as the percentage of germinated seeds.

The percentage of reduction of carcinogenic risk assessment (RCRA) of the PAH present in the creosote soil was based on the toxic equivalency factors (TEF) of PAH for environmental exposure (Nisbet and LaGoy 1992) and was determined as follows:

$$RCRA(\%) = \frac{\sum_{i=1}^{13} [PAH_i]_{to} \cdot DR \cdot TEF_i}{\sum_{i=1}^{13} [PAH_i]_{to} \cdot TEF_i} \cdot 100$$

where  $[PAH_i]_{to}$  is the initial concentration of PAH, DR the degradation rate and TEF the toxic equivalency factor (Table 3).

## 2.6 Statistical analysis

All statistical tests were carried out using the IBM SPSS Statistics v21 software package. One-way analysis of variance was carried out after performing the Levene variance homogeneity test. To compare the differences between treatments, the Tukey or Games–Howell post hoc test (according to variance homogeneity) at  $p < 0.05$  was used. Two-way ANOVA was used to investigate interaction between AC and C.

## 3. Results and Discussion

### 3.1. Effect of soil amendments on soluble metals and As in the mining soil

Pore water pH, EC and DOC of the 7 microcosms tested in the mining soil, measured in pore water at 5 weeks of incubation, are depicted in Figure 1 (A, B and C). Unamended soil was strongly acid and presented low DOC concentration. AC and compost materials are characterized by their alkaline character, what can provoke an increase in soil pH and, consequently, a decrease in metals mobility. In the studied cases, the application of AC had a minimal effect on soil pH, that could be due to the low dose of AC applied (1%, w:w; *i.e.* aprox. 25 t ha<sup>-1</sup>) with respect to previous published works (Beesley et al. 2013, 2014; Elouear et al. 2014). However, the combination of these AC with compost at a dose of 2% (aprox. 50 t ha<sup>-1</sup>) significantly increased soil pH to values up to 4.2, which was probably related to compost physico-chemical properties, such as high total concentrations of basic cations, alkaline pH, high buffer capacity in the acid range (345 mmol<sub>c</sub> kg<sup>-1</sup> per pH unit) and a content of 7% of CaCO<sub>3</sub> and C/N ratio of 14 (Albuquerque et al. 2011). This agrees with that previously observed by Karer et al. (2015) when mixed three different biochars and compost. The EC followed a similar trend, as the single



application of AC did not increase it, but the mixture of AC+C increased EC more than 2.5-fold with respect to the unamended soil (Fig. 1B). All treatments significantly decreased DOC (Fig. 1C), suggesting that AC were able to adsorb soluble OM.

Table 4 collects metals and As concentration in pore water extracted from the mining soil-remediation microcosms. The application of AC as single amendment had in general limited effects on metal(loid)s solubility, but according to two-way ANOVA analysis, the three AC tested differed in their efficiency to immobilize metals and As, being AC3 the most effective AC ( $P<0.05$ ), as the concentration of As, Al, Cd, Cu, Fe and Pb significantly decreased after 35 days of incubation. It may be attributed to the higher adsorption capacity of AC3 that presented the largest  $A_{Ext}$ ,  $V_{mesoropores}$  and desorption of  $CO_2$  (related with surficial oxygen-containing functional groups) (Table 2). Besides, addition of AC3 led to a slightly increase in soil pH, likely due to its higher pH slurry. A higher pH can enhance metals adsorption capacity of AC and soil matrix components by deprotonation of functional groups, such as carboxyl or hydroxyl, and can even induce metal precipitation due to the formation of insoluble hydroxides (Karer et al. 2015). Since soluble Fe was also lower in the AC3-treated soil, we hypothesize that iron (hydr)oxides precipitated in this soil or AC surface may have enhanced As retention, resulting in lower soluble concentration of this metalloid (Table 4).

The combination of AC+C generally resulted in lower concentration of soluble metal(loid)s in pore water, compared to the control and the only-AC treatments, but no significant differences between AC+C treatments were found (Table 4). The percentages of reduction of metal concentration after the application of AC+C were between 75 and 99% with respect to the unamended soil. Two-way ANOVA test demonstrated the significant ( $P<0.001$ ) effect of AC+C application with respect to AC treatments. This fact may be attributed to the combination of two effects; on the one hand, an increment in soil pH (Fig. 1-A) would induce metals precipitation and thus reduce their solubility and on the other hand, the OM of the compost used, which is rich in humic substances, can interact with heavy metals by chelation, adsorption or retention in its exchange complex, reducing their mobility (Clemente et al. 2015).

The chemical behavior of As in soils is different to metals because it is usually present as oxyanions (arsenite/arsenate). The application of organic amendments to multi-contaminated soils generally results in an increase in soil pH, which can increase As mobility (Beesley et al. 2014). Furthermore, competition of dissolved organic matter and arsenate for sorption sites has been reported, leading to As desorption

(Bauer and Blodau 2006; Arco-Lázaro et al. 2016). However, soluble As was reduced in a lower extent, ~11, 9 and 33% by AC1+C, AC2+C and AC3+C, respectively, with respect to the unamended soil, being the main As species found was As (V) (up to 93%, data not shown). Given that an increase in DOC (Fig. 1) and As (Table 4) in soil pore water was not found in the AC+C-treated soils, it can be assumed that addition of AC together with C prevented As mobilization provoked by addition of compost, which has been previously reported by other authors (Pardo et al. 2011; Clemente et al. 2012; Beesley et al. 2014).

Mineralization of OM can be expected in the long-term, especially given some of the physico-chemical characteristics of the tested soil, such as high content of sand, low pH and high concentrations of potentially toxic trace elements (Albuquerque et al., 2011; Pardo et al., 2011). If metals stabilization was mainly provoked by the addition of OM from compost, it can be expected that mineralization of this OM results in an increase in metals mobility over time. This effect could be mitigated by the addition of AC since, as recently reported by Ouyang et al. (2014) for biochar, the high surface specific area of this material can enhance organic matter adsorption, thus reducing its availability for microbial degradation.

### 3.2. Degradation and availability of PAH in the creosote soil

The PAH degradation by natural attenuation (S), AC and AC+C application was tested on creosote polluted soil from a wood preservation plant. Figure 1 (D, E and F) shows values of pH, EC and DOC of the 7 microcosms tested in the creosote soil, measured in water extracts (1:10 w:v) at 5 weeks of incubation. In general, the AC or AC+C treatments in the creosote polluted soil did not modify soil pH, except the AC3+C treatment, which significantly increased it (Fig. 1-D). The EC increased in all the AC+C-treated soils, but not in the AC-treated ones. DOC was significantly reduced (24–44%) by the application of AC. However AC+C treatments did not modify DOC concentration in the creosote soil. Therefore, AC seemed to be able to adsorb soluble OM, as happened in the mining soil.

Results of total PAH concentration from the initial soil and the degradation percentage of each remediation treatment are in Table 3. The degradation was calculated by difference between initial and final total concentration of each treatment. Total PAH concentration was  $3392 \pm 190 \text{ mg kg}^{-1}$ . The soil was mainly contaminated by PAH with 3-rings (85%), although the concentration of PAH with 4 and 5,6-rings was very high (398 and  $98.1 \text{ mg kg}^{-1}$  respectively).

The unamended soil (S) reached 56% of total PAH ( $\Sigma$ PAH) degradation because of the relatively high degradation rate of PAH with 3-rings. The degradation of PAH with 4- and 5,6- rings was very low (1%). The reason might be that the degradation of PAH is mainly carried out by bacteria, which are effective to degrade low molecular weight (LMW) PAH but whose potential to degrade HMW-PAH is limited (García-Delgado et al. 2015b). In addition, the high PAH degradation efficiency achieved in the unamended soil likely occurred due to the ability of indigenous microbial community to adapt to the actual polluted environment and degrade targeted contaminants (Lladó et al. 2015; Lukic et al. 2016; Lukić et al. 2017). Degradation of PAH was previously described in an unamended soil due to the high degradation of LMW-PAH, despite unappreciable degradation of 4- and 5,6-rings PAH (Lukić et al. 2017).

The application of AC did not increase the degradation of total ( $\Sigma$ PAH) and 3-rings PAH. However, these amendments significantly increased the degradation rate of high molecular weight (HMW) PAH. No significant differences were obtained between the three AC applied. The percentage of 4-rings and 5,6-rings PAH in the AC-treated soils was 19 – 21% and 10–26%, respectively. Previous works showed the enhancement of HMW-PAH degradation by application of biochar (García-Delgado et al. 2015a; Oleszczuk et al. 2016) because this material can modify the microbial community structure of the soil in favor of gram positive bacteria or mycobacterium that degrade recalcitrant organic compounds and aromatic carbon in soils (Anyika et al. 2015). Stefaniuk et al. (2017) reported enhanced HMW-PAH degradation in field conditions by application of biochar because it provided an additional source of nutrients, which stimulated microbial activity, and attached bacteria on biochar surface. Therefore, amendments with high recalcitrant carbon such as biochar or activated carbon seem to be effective to promote the degradation of HMW-PAH.

Bioavailable PAH extracted by HPCD is the fraction of these contaminants that is accessible to microorganisms (Stokes et al. 2005). Results of available PAH concentrations in each microcosm are shown in Table 5. The  $\Sigma$ PAH available fraction in the initial soil was 2356 mg kg<sup>-1</sup>, which means 69% of the total PAH concentration. The most available group was PAH with 3-rings (74% of total 3-rings PAH) followed by PAH with 4-rings (50% of total 4-rings PAH) and finally by PAH with 5,6-rings (12% of total 5,6-rings PAH). The initial high available concentration of 3-rings PAH (2143 mg kg<sup>-1</sup> in S<sub>t0</sub>, Table 5) was the main reason of their great degradation because the availability of the organic pollutants is a

key factor in the biodegradation process. The concentration of 3-rings PAH degraded along the assay in the untreated soil was 88% of its initial available fraction.

The  $\Sigma$ PAH availability was about 65% lower in the unamended microcosm (S) than in the initial soil after 35 days of incubation (Fig. 2). This result was similar to the AC treatments (AC1 64%, AC2 57%, AC3 65%) and no significant differences between the unamended soil and AC treatments were observed. The decrease of PAH availability in the unamended soil at the end of the assay was because of the high degradation of 3-rings PAH and the stabilization of HMW-PAH. It can be observed in Figure 2 that the decrease of 3-rings PAH availability is very similar to the degradation percentage, which means that most of the available fraction of LMW-PAH has been degraded in the unamended soil. In contrast, the degradation of 4- and 5,6-rings PAHs is very low with respect to the decrease of their availability and thus it can be assumed that most of the HMW-PAH have been adsorbed onto the soil but not degraded.

Two-way ANOVA showed that the kind of AC significantly affected PAH degradation, irrespective of the number of rings (Table 3). AC1 and AC2 showed similar behavior towards PAH degradation and immobilization. They were able to enhance the degradation of HMW-PAH and to simultaneously decrease the concentration of available 5,6-rings PAH because the reduction of available concentration (90% by AC1 and AC2) was higher than their respective degradation percentages (10 and 25%, respectively; Fig. 2). The effects appeared contradictory but the increment of the degradation can be explained because the decrease of HMW-PAH availability, which are the most toxic PAH (Nisbet and LaGoy 1992), decreased the soil toxicity to microorganisms, thereby increasing the microbial activity and degradation (Anyika et al. 2015). AC3 was able to enhance the degradation of both, 4- and 5,6-rings PAH (Table 3), and simultaneously adsorb 3-rings PAH (Fig. 2). The other AC (AC1 and AC2) led to similar decrease in 3-rings PAH availability and degradation. Therefore, in AC1- and AC2-treated soils the immobilization of 3-rings PAH was very low and the decrease in their availability was due to biodegradation. The different behavior of the three AC towards LMW- and HMW-PAH bioavailability was demonstrated by the two-way ANOVA (Table 5). The kind of AC affected significantly the bioavailable concentration of 3- and 4-rings PAH but not the concentration of 5,6-rings PAH. The differences in the immobilization of 3-rings PAH by AC3 may be because of the differences in structural properties of the three AC (Table 2), since external area and mesopores volume in AC1 and AC2 was lower than in AC3. The relatively low impact that the three AC had on  $\Sigma$ PAH availability reduction can be explained by adsorption of DOC (Fig. 1-F) or oils that can fill total or partially the PAH adsorption

positions on AC surface (Brändli et al. 2008; Marchal et al. 2013b; Kołtowski et al. 2016) and by a co-solvent effect due to the presence of oils (organic solvents) in the soil that in consequence may reduce the effectivity of AC towards PAH adsorption. Cabal et al. (2009) reported the tendency of naphthalene to remain in solution when organic solvents were used, thereby reducing the overall amount of PAH adsorbed on AC. Another possible factor that can decrease the efficiency of AC to reduce PAH availability is the neutral-basic pH of the soil (Table 1) and basic character of AC2 and AC3 (Table 2). Basic pH produces negative charge of the AC surface and therefore increases the polarity of the AC and the repulsive electrostatic forces, which inhibits adsorption of hydrophobic compounds. Monsalvo et al. (2012) reported this phenomenon for 4-chlorophenol adsorption by pyrolyzed sewage sludge. Finally, the low dose applied (1%) with respect to previous works (Brändli et al. 2008; Jakob et al. 2012; Brennan et al. 2014) could minimize the decrease of PAH availability. However, the AC dose used in this work would be equivalent to a field application rate of 25 t ha<sup>-1</sup>, so a higher amount could not be feasible in real applications due to the relative high cost of AC (Brennan et al. 2014).

The combination of AC and compost (AC1+C and AC2+C) promoted the degradation of PAH with 3- and 4-rings and ΣPAH. These two treatments were the most effective to enhance ΣPAH degradation (77 and 80% respectively) (Table 3). In contrast, in microcosm AC3+C, the degradation of either any individual PAH or ΣPAH was not increased with respect to AC3.

The addition of C significantly affected the degradation of PAH, according to two-way ANOVA results (Table 3). Probably the main reason was the biostimulation of soil microorganisms by compost application. In addition, the carbon of this compost is very persistent in soil (Pardo et al. 2011) and it has been reported that stabilized organic amendments obtained higher degradation of PAH than the non-stabilized ones (Sayara et al. 2010). The combination of AC1+C and AC2+C enhanced the degradation of PAH with respect to AC1 and AC2, mainly 3 and 4-rings PAH (Fig. 2). However, no significant differences were found in the percentage of PAH degradation between AC3+C and AC3 treatments. Therefore, benefits in degradation effectiveness resulting from the addition of C were also affected by the kind of AC applied.

AC1+C, AC2+C and AC3+C treatments decreased the available fraction of ΣPAH by 81, 83 and 58%, respectively, compared to the untreated soil, S (Table 5). However, this decrease was statistically significant only in the case of AC1+C and AC2+C. These treatments were able to significantly reduce the

available concentration of many individual PAH with 3 or 4-rings, such as Ant, Fla, Py, BaA and Chr, but not of  $\Sigma$ 5,6-rings PAH. The mechanism by which PAH availability was reduced in AC+C treatments was different for 3-, 4-rings PAH and 5,6-rings PAH. No significant differences were found between the decrease of availability and degradation for 3-, 4 rings PAH in AC+C treatments, but for 5,6-rings PAH, the decrease of availability was higher than the degradation (Fig. 2). Therefore, it can be assumed that the decrease of 3- and 4-rings PAH availability was due to degradation of the available fraction, whereas the decrease of 5,6-rings PAH availability was mainly due to adsorption onto the soil/amendment composite.

### 3.3 Eco-toxicity assessment

Soil toxicity was assessed by the *V. fischeri* luminescence inhibition test and *L. sativa* germination index for heavy metal and PAH polluted soils additionally, the RCRA was assessed for the PAH polluted soil (Table 6).

Based on the low EC<sub>50</sub> values obtained, *V. fischeri* was highly sensitive to the mining soil leachates. The addition of AC1 and AC2 did not reduce toxicity towards this organism with respect to the unamended soil, as shown by similar EC<sub>50</sub> values (Table 6). However, AC3 and the combination of AC with compost (AC+C treatments) slightly alleviated toxicity of soil leachates. Since these treatments reduced heavy metals solubility, soil pore water composition seemed to greatly affect the luminescence of *V. fischeri*, as suggested by Alvarenga et al. (2009).

The application of AC as single amendment was not effective to produce germination of *L. sativa* in the mining soil. Nevertheless, the combination of AC and compost reached germination indexes higher than 85%. So the combination of AC and compost was very effective to reduce soil toxicity for seed germination likely as a consequence of a simultaneous reduction of acidity and heavy metals solubility in all the AC+C treatments. However, it has been suggested that the supply of OM and nutrients by compost is the most likely reason for an increase in lettuce germination (Pardo et al. 2014). Beesley et al. (2014) obtained similar results of mining soil detoxification towards *V. fischeri* luminescence and *Lolium perenne* germination after soil amended with a biochar or combination of compost and biochar. In view of these results, the combination of AC and compost helps to alleviate soil and leachates toxicity in the tested mining soil, being AC3+C the treatment that showed the most promising results.

In the case of PAH polluted soil, all treatments were effective to reduce the luminescence inhibition with respect to the unamended soil (Table 6). The lack of significant differences between treatments could be because the low solubility of PAH in water and the decrease of PAH content and availability in all the amended soils. The application of AC with or without compost significantly increased the germination of *L. sativa* and no differences were found between the AC and AC+C treatments. It is noteworthy the great increment of germination success of *L. sativa* in AC treatments despite their relative low impact on PAH degradation. In this line, Brennan et al. (2014) observed that AC improved plant biomass production, while consistently reducing PAH bioavailable fractions and measured uptake by plants.

The reduction of the carcinogenic risk assessment of treatments AC2 and AC3 with and without compost (26–35%) was higher than S (4%) and AC1 (14%). However the highest RCRA was achieved for the AC2+C treatment (35%). The parameter RCRA combines the degradation rate of each PAH and its respective toxic equivalent factor. Hence, this parameter shows the relative detoxification better than the degradation of individual or total PAH. However, PAH availability was not included in the estimation of RCRA and thus it could be higher for treatments that reduce the available fraction of the most toxic PAH such as HMW-PAH, specially BaP or DBaH.

## 5. Conclusions

Activated carbon effectively reduces metal(loid)s mobility and enhances HMW-PAH degradation, but the co-application of AC+C shows optimum effects. The application of activated carbon mixed with olive mill waste compost further promotes the immobilization of heavy metals and As and reduces acidity in the mining soil. In the creosote-contaminated soil, the co-application of AC+C further increases PAH degradation and reduces their availability. In good agreement, the ecotoxicological tests clearly demonstrate that the application of activated carbon and compost mixtures enhances the remediation efficiency of activated carbons. Overall, the co-application of activated carbon and compost is more efficient than the single addition of activated carbon at decreasing toxicological risk and improving soil health.

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Table 1: Basic characterization of mine and creosote soils and the compost. Mean values  $\pm$  standard deviation (n = 3).

	Mining soil	Creosote soil	Compost
pH	3.0 $\pm$ 0.02	7.78 $\pm$ 0.012	8.8 $\pm$ 0.01
EC (dS m <sup>-1</sup> )	0.22 $\pm$ 0.05	0.88 $\pm$ 0.24	6.1 $\pm$ 0.2
OM (%)	0.3 $\pm$ 0.05	8.1 $\pm$ 0.4	70.2 $\pm$ 3
CaCO <sub>3</sub> (%)	n.d.	38 $\pm$ 3	n.a.
Sand (%)	90	7.5 $\pm$ 2.3	n.a.
Silt (%)	3.8	39 $\pm$ 4	n.a.
Clay (%)	6.2	54 $\pm$ 2	n.a.
As (mg kg <sup>-1</sup> )	2360 $\pm$ 203	11.9 $\pm$ 0.8	n.d.
Cd (mg kg <sup>-1</sup> )	10.5 $\pm$ 4.3	0.20 $\pm$ 0.06	n.d.
Cu (mg kg <sup>-1</sup> )	568 $\pm$ 18	159 $\pm$ 17	48 $\pm$ 1
Fe (g kg <sup>-1</sup> )	28.82 $\pm$ 3.6	16.7 $\pm$ 1.8	1.62 $\pm$ 0.1
Pb (mg kg <sup>-1</sup> )	891 $\pm$ 23	11.8 $\pm$ 1.1	36 $\pm$ 2
Zn (mg kg <sup>-1</sup> )	2330 $\pm$ 430	115 $\pm$ 9	141 $\pm$ 4

n.a. not analysed

n.d. not detected.

Table 2: Characterization and porous structure of the activated carbons: BET area, external area, micropore and mesopore Volume, elemental composition, ashes, CO<sub>2</sub> and CO adsorption capacity and pH slurry (1:10, v:w). AC1: Chemviron, AC2: Merck, AC3: Norit.

	AC1	AC2	AC3
A <sub>BET</sub> (m <sup>2</sup> g <sup>-1</sup> )	1392	917	1065
A <sub>Ext</sub> (m <sup>2</sup> g <sup>-1</sup> )	110	119	162
V <sub>micropores</sub> (cm <sup>3</sup> g <sup>-1</sup> )	0.549	0.332	0.391
V <sub>mesopores</sub> (cm <sup>3</sup> g <sup>-1</sup> )	0.170	0.118	0.270
C (%)	84.3	89.5	86.7
H (%)	0.25	0.50	1.0
N (%)	1.3	0.53	0.58
S (%)	0.09	0.11	0.78
O (%) <sup>a</sup>	9.26	4.66	2.54
Ashes (%)	4.8	4.7	8.4
CO <sub>2</sub> (μmol g <sup>-1</sup> )	137	91	330
CO (μmol g <sup>-1</sup> )	357	893	782
pH slurry	6.2	8.1	10.4

<sup>a</sup> Calculated by difference between the sum of C, H, N and S and the ash content.

Table 3: Total PAH concentration (mean  $\pm$  standard deviation) of the creosote initial soil ( $S_0$ ) and PAH degradation percentage of each remediation treatments (S: control soil; AC: activated carbon (1%); AC+C: activated carbon (1%) + compost (2%)) after 35 days of incubation.

	$S_0$	TEF <sup>†</sup>	S	AC1	AC2	AC3	AC1+C	AC2+C	AC3+C	<i>Two-Way ANOVA</i>		
	(mg kg <sup>-1</sup> )		PAH degradation (%)							AC	C	AC x C
<b>Ace</b>	1131 $\pm$ 114	0.001	54 <sup>ab</sup>	57 <sup>abc</sup>	41 <sup>a</sup>	41 <sup>a</sup>	77 <sup>bc</sup>	83 <sup>c</sup>	56 <sup>abc</sup>	**	***	N.S.
<b>Flu</b>	961 $\pm$ 49	0.001	70 <sup>ab</sup>	70 <sup>ab</sup>	57 <sup>a</sup>	54 <sup>a</sup>	84 <sup>b</sup>	84 <sup>b</sup>	70 <sup>ab</sup>	**	***	N.S.
<b>Phe</b>	668 $\pm$ 20	0.001	82 <sup>ab</sup>	81 <sup>ab</sup>	71 <sup>a</sup>	70 <sup>a</sup>	89 <sup>b</sup>	89 <sup>b</sup>	80 <sup>ab</sup>	**	***	N.S.
<b>Ant</b>	137 $\pm$ 6	0.01	40 <sup>a</sup>	52 <sup>ab</sup>	44 <sup>a</sup>	41 <sup>a</sup>	72 <sup>b</sup>	72 <sup>b</sup>	53 <sup>ab</sup>	*	***	N.S.
<b>Fla</b>	130 $\pm$ 4	0.001	2 <sup>a</sup>	26 <sup>bc</sup>	19 <sup>b</sup>	25 <sup>bc</sup>	62 <sup>d</sup>	64 <sup>d</sup>	38 <sup>c</sup>	*	***	**
<b>Py</b>	148 $\pm$ 4	0.001	1 <sup>a</sup>	17 <sup>ab</sup>	10 <sup>ab</sup>	18 <sup>ab</sup>	59 <sup>c</sup>	65 <sup>c</sup>	27 <sup>b</sup>	**	***	***
<b>BaA</b>	53.1 $\pm$ 1.2	0.1	2 <sup>a</sup>	15 <sup>ab</sup>	20 <sup>ab</sup>	21 <sup>ab</sup>	55 <sup>c</sup>	65 <sup>c</sup>	27 <sup>b</sup>	*	***	**
<b>Chr</b>	67.2 $\pm$ 2.7	0.01	2 <sup>a</sup>	14 <sup>a</sup>	15 <sup>a</sup>	19 <sup>a</sup>	45 <sup>b</sup>	54 <sup>b</sup>	18 <sup>a</sup>	*	***	**
<b>BbF</b>	49.8 $\pm$ 2.1	0.1	1 <sup>a</sup>	9 <sup>ab</sup>	16 <sup>ab</sup>	17 <sup>ab</sup>	11 <sup>ab</sup>	26 <sup>b</sup>	9 <sup>ab</sup>	N.S.	N.S.	N.S.
<b>BkF</b>	14.0 $\pm$ 0.5	0.1	2 <sup>a</sup>	14 <sup>ab</sup>	29 <sup>b</sup>	25 <sup>b</sup>	16 <sup>ab</sup>	29 <sup>b</sup>	25 <sup>b</sup>	*	N.S.	N.S.
<b>BaP</b>	15.3 $\pm$ 0.5	1	2 <sup>a</sup>	16 <sup>ab</sup>	32 <sup>b</sup>	29 <sup>ab</sup>	13 <sup>ab</sup>	30 <sup>b</sup>	27 <sup>ab</sup>	*	N.S.	N.S.
<b>DBahA</b>	0.62 $\pm$ 0.12	5	3 <sup>a</sup>	0 <sup>a</sup>	51 <sup>b</sup>	52 <sup>bc</sup>	11 <sup>ab</sup>	46 <sup>bc</sup>	55 <sup>c</sup>	*	N.S.	N.S.
<b>BghiP</b>	8.88 $\pm$ 0.48	0.01	1 <sup>a</sup>	13 <sup>ab</sup>	33 <sup>b</sup>	28 <sup>b</sup>	4 <sup>a</sup>	20 <sup>ab</sup>	14 <sup>ab</sup>	*	*	N.S.
<b>IcdP</b>	9.51 $\pm$ 0.54	0.1	0 <sup>a</sup>	5 <sup>a</sup>	26 <sup>b</sup>	22 <sup>b</sup>	2 <sup>a</sup>	21 <sup>b</sup>	20 <sup>b</sup>	**	N.S.	N.S.
<b><math>\Sigma</math>3-rings</b>	2896 $\pm$ 176		65 <sup>ab</sup>	67 <sup>ab</sup>	53 <sup>a</sup>	52 <sup>a</sup>	82 <sup>c</sup>	84 <sup>c</sup>	66 <sup>ab</sup>	**	***	N.S.
<b><math>\Sigma</math>4-rings</b>	398 $\pm$ 11		1 <sup>a</sup>	19 <sup>b</sup>	15 <sup>ab</sup>	21 <sup>b</sup>	57 <sup>c</sup>	63 <sup>c</sup>	29 <sup>b</sup>	**	***	***
<b><math>\Sigma</math>5,6-rings</b>	98.1 $\pm$ 4.3		1 <sup>a</sup>	10 <sup>ab</sup>	25 <sup>b</sup>	22 <sup>b</sup>	10 <sup>ab</sup>	26 <sup>b</sup>	21 <sup>b</sup>	*	N.S.	N.S.
<b><math>\Sigma</math>PAH</b>	3392 $\pm$ 190		56 <sup>a</sup>	60 <sup>a</sup>	48 <sup>a</sup>	47 <sup>a</sup>	77 <sup>b</sup>	80 <sup>b</sup>	60 <sup>a</sup>	**	***	N.S.

Different letters indicate significant differences between treatments (n = 4; Tukey's test,  $p < 0.05$ ). Analysis of variance (two-way ANOVA) was performed to evaluate the effect of each factor (AC and C) and their interaction. N.S.: not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

<sup>†</sup> Toxic Equivalence Factors (Nisbet and LaGoy 1992).

Table 4: Concentration (mg L<sup>-1</sup>) of metals and As (mean  $\pm$  standard deviation;  $n = 4$ ) in pore water extracted from microcosms made with the mining soil after 35 days of incubation.

	Al	As	Cd	Cu	Fe	Pb	Zn
S	90 $\pm$ 4 <sup>c</sup>	0.64 $\pm$ 0.01 <sup>c</sup>	2.97 $\pm$ 0.08 <sup>d</sup>	42.12 $\pm$ 1.50 <sup>d</sup>	12.07 $\pm$ 0.68 <sup>c</sup>	0.378 $\pm$ 0.010 <sup>d</sup>	326 $\pm$ 13 <sup>b</sup>
AC1	80 $\pm$ 2 <sup>c</sup>	0.57 $\pm$ 0.02 <sup>bc</sup>	2.69 $\pm$ 0.04 <sup>c</sup>	25.40 $\pm$ 0.36 <sup>c</sup>	9.47 $\pm$ 0.08 <sup>c</sup>	0.273 $\pm$ 0.005 <sup>c</sup>	305 $\pm$ 5 <sup>b</sup>
AC2	84 $\pm$ 4 <sup>c</sup>	0.57 $\pm$ 0.02 <sup>bc</sup>	3.25 $\pm$ 0.04 <sup>c</sup>	38.23 $\pm$ 1.31 <sup>d</sup>	10.91 $\pm$ 0.59 <sup>c</sup>	0.355 $\pm$ 0.009 <sup>d</sup>	354 $\pm$ 18 <sup>b</sup>
AC3	53 $\pm$ 1 <sup>b</sup>	0.36 $\pm$ 0.04 <sup>a</sup>	2.02 $\pm$ 0.07 <sup>b</sup>	17.72 $\pm$ 0.47 <sup>b</sup>	2.78 $\pm$ 0.14 <sup>b</sup>	0.213 $\pm$ 0.005 <sup>b</sup>	279 $\pm$ 9 <sup>b</sup>
AC1+C	1.2 $\pm$ 0.1 <sup>a</sup>	0.51 $\pm$ 0.04 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.18 $\pm$ 0.02 <sup>a</sup>	0.040 $\pm$ 0.001 <sup>a</sup>	33 $\pm$ 4 <sup>a</sup>
AC2+C	1.7 $\pm$ 0.7 <sup>a</sup>	0.52 $\pm$ 0.04 <sup>bc</sup>	0.28 $\pm$ 0.06 <sup>a</sup>	0.17 $\pm$ 0.05 <sup>a</sup>	0.29 $\pm$ 0.05 <sup>a</sup>	0.043 $\pm$ 0.003 <sup>a</sup>	43 $\pm$ 6 <sup>a</sup>
AC3+C	1.1 $\pm$ 0.4 <sup>a</sup>	0.48 $\pm$ 0.02 <sup>ab</sup>	0.18 $\pm$ 0.04 <sup>a</sup>	0.09 $\pm$ 0.03 <sup>a</sup>	0.11 $\pm$ 0.03 <sup>a</sup>	0.040 $\pm$ 0.004 <sup>a</sup>	24 $\pm$ 3 <sup>a</sup>
<i>Two-way ANOVA</i>							
AC	***	**	***	***	***	***	***
C	***	N.S.	***	***	***	***	***
AC x C	***	*	***	***	***	***	*

Different letters indicate significant differences between treatments (Tukey's test,  $p < 0.05$ ). Analysis of variance (two-way ANOVA) was performed to evaluate the effect of each factor (AC and C) and their interaction. N.S.: not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



Table 5: PAH available concentration (mg kg<sup>-1</sup>;  $n = 4$ ) of the creosote initial soil (S<sub>t0</sub>) and the different remediation treatments (S: control soil; AC: activated carbon (1%); AC+C: activated carbon (1%) + compost (2%)) after 35 days of incubation.

	S <sub>t0</sub>	S	AC1	AC2	AC3	AC1+C	AC2+C	AC3+C	<i>Two way ANOVA</i>		
									AC	C	AC x C
<b>Ace</b>	799 <sup>c</sup>	359 <sup>ab</sup>	352 <sup>ab</sup>	451 <sup>b</sup>	185 <sup>a</sup>	202 <sup>ab</sup>	145 <sup>ab</sup>	394 <sup>ab</sup>	N.S.	*	***
<b>Flu</b>	753 <sup>c</sup>	171 <sup>ab</sup>	208 <sup>ab</sup>	251 <sup>ab</sup>	301 <sup>b</sup>	113 <sup>a</sup>	123 <sup>a</sup>	274 <sup>b</sup>	**	**	N.S.
<b>Phe</b>	498 <sup>c</sup>	63.5 <sup>ab</sup>	73.4 <sup>ab</sup>	102 <sup>ab</sup>	116 <sup>b</sup>	43.7 <sup>a</sup>	37.8 <sup>a</sup>	97.3 <sup>ab</sup>	*	*	N.S.
<b>Ant</b>	93.9 <sup>c</sup>	50.8 <sup>b</sup>	39.6 <sup>ab</sup>	39.7 <sup>ab</sup>	49.4 <sup>ab</sup>	23.3 <sup>ab</sup>	20.1 <sup>a</sup>	45.1 <sup>ab</sup>	*	*	N.S.
<b>Fla</b>	75.1 <sup>b</sup>	62.6 <sup>b</sup>	53.2 <sup>ab</sup>	50.0 <sup>ab</sup>	56.1 <sup>b</sup>	25.3 <sup>a</sup>	26.3 <sup>a</sup>	52.5 <sup>ab</sup>	*	**	N.S.
<b>Py</b>	70.8 <sup>b</sup>	64.9 <sup>b</sup>	66.6 <sup>b</sup>	81.7 <sup>b</sup>	69.3 <sup>b</sup>	28.3 <sup>a</sup>	25.6 <sup>a</sup>	72.4 <sup>b</sup>	**	***	***
<b>BaA</b>	27.7 <sup>b</sup>	23.7 <sup>b</sup>	22.5 <sup>b</sup>	21.8 <sup>b</sup>	23.4 <sup>b</sup>	1.29 <sup>a</sup>	6.16 <sup>a</sup>	18.9 <sup>b</sup>	***	***	**
<b>Chr</b>	27.4 <sup>c</sup>	20.1 <sup>bc</sup>	21.4 <sup>bc</sup>	24.9 <sup>c</sup>	25.7 <sup>c</sup>	2.04 <sup>a</sup>	10.8 <sup>ab</sup>	23.3 <sup>bc</sup>	**	***	*
<b>BbF</b>	8.09 <sup>c</sup>	3.11 <sup>ab</sup>	0.38 <sup>a</sup>	0.96 <sup>a</sup>	6.86 <sup>bc</sup>	0.54 <sup>a</sup>	0.53 <sup>a</sup>	1.40 <sup>a</sup>	N.S.	N.S.	*
<b>BkF</b>	1.90 <sup>c</sup>	0.86 <sup>b</sup>	0.56 <sup>ab</sup>	0.05 <sup>a</sup>	0.38 <sup>ab</sup>	0.20 <sup>ab</sup>	0.12 <sup>ab</sup>	<0.01 <sup>a</sup>	N.S.	N.S.	N.S.
<b>BaP</b>	1.19 <sup>b</sup>	0.17 <sup>a</sup>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	N.S.	N.S.	N.S.
<b>DBhaA</b>	<0.01 <sup>a</sup>	<0.01 <sup>a</sup>	0.17 <sup>abc</sup>	0.09 <sup>ab</sup>	0.26 <sup>abc</sup>	0.44 <sup>c</sup>	0.32 <sup>bc</sup>	0.03 <sup>ab</sup>	**	N.S.	**
<b>BghiP</b>	0.13	<0.01	0.04	<0.01	<0.01	0.49	<0.01	<0.01	N.S.	N.S.	N.S.
<b>IcdP</b>	<0.01	<0.01	<0.01	<0.01	<0.01	0.24	<0.01	0.35	N.S.	N.S.	N.S.
<b>Σ3rings</b>	2143 <sup>c</sup>	644 <sup>b</sup>	674 <sup>b</sup>	844 <sup>b</sup>	651 <sup>b</sup>	382 <sup>a</sup>	326 <sup>a</sup>	810 <sup>b</sup>	**	***	***
<b>Σ4rings</b>	201 <sup>b</sup>	171 <sup>b</sup>	164 <sup>b</sup>	178 <sup>b</sup>	174 <sup>b</sup>	57.0 <sup>a</sup>	68.9 <sup>a</sup>	167 <sup>b</sup>	**	***	**
<b>Σ5,6rings</b>	11.3 <sup>c</sup>	4.14 <sup>ab</sup>	1.11 <sup>a</sup>	1.10 <sup>a</sup>	7.49 <sup>bc</sup>	1.91 <sup>ab</sup>	0.96 <sup>a</sup>	1.77 <sup>ab</sup>	N.S.	N.S.	*
<b>ΣPAH</b>	2356 <sup>c</sup>	820 <sup>b</sup>	839 <sup>b</sup>	1023 <sup>b</sup>	833 <sup>b</sup>	441 <sup>a</sup>	396 <sup>a</sup>	979 <sup>b</sup>	**	***	***

Different letters indicate significant differences between treatments (Tukey's HSD test,  $p < 0.05$ ). Analysis of variance (two-way ANOVA) was performed to evaluate the effect of each factor (AC and C) and their interaction. N.S.: not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Table 6: EC<sub>50</sub><sup>a</sup> of *V. fischeri* and germination index of *L. sativa* in aqueous extracts of mining and creosote polluted soil and RCRA<sup>b</sup> of creosote polluted soil for each treatment (S: control soil; AC: activated carbon (1%); AC+C: activated carbon (1%) + compost (2%)) after 35 days of incubation. Data are mean ( $n = 4$ ).

Microcosm	Mine Soil		Creosote Soil		
	EC <sub>50</sub> (%)	Germination (%)	EC <sub>50</sub> (%)	Germination (%)	RCRA (%)
S	13	0 <sup>a</sup>	87	18 <sup>a</sup>	4 <sup>a</sup>
AC1	11	0 <sup>a</sup>	100	83 <sup>b</sup>	14 <sup>ab</sup>
AC2	13	0 <sup>a</sup>	-	78 <sup>b</sup>	29 <sup>bc</sup>
AC3	20	0 <sup>b</sup>	-	68 <sup>b</sup>	27 <sup>bc</sup>
AC1+C	20	85 <sup>b</sup>	-	90 <sup>b</sup>	18 <sup>abc</sup>
AC2+C	22	98 <sup>c</sup>	-	75 <sup>b</sup>	35 <sup>c</sup>
AC3+C	34	95 <sup>bc</sup>	-	85 <sup>b</sup>	26 <sup>bc</sup>

<sup>a</sup> Percentage of soil extract compared to non-toxic control that caused a 50% inhibition in luminescence.

<sup>b</sup> Reduction of carcinogenic risk assessment expressed as percentage was based on toxic equivalency factors proposed by Nisbet and LaGoy (1992).

Different letters indicate significant differences between microcosms ( $n = 4$ ; Tukey's test,  $p < 0.05$ ).

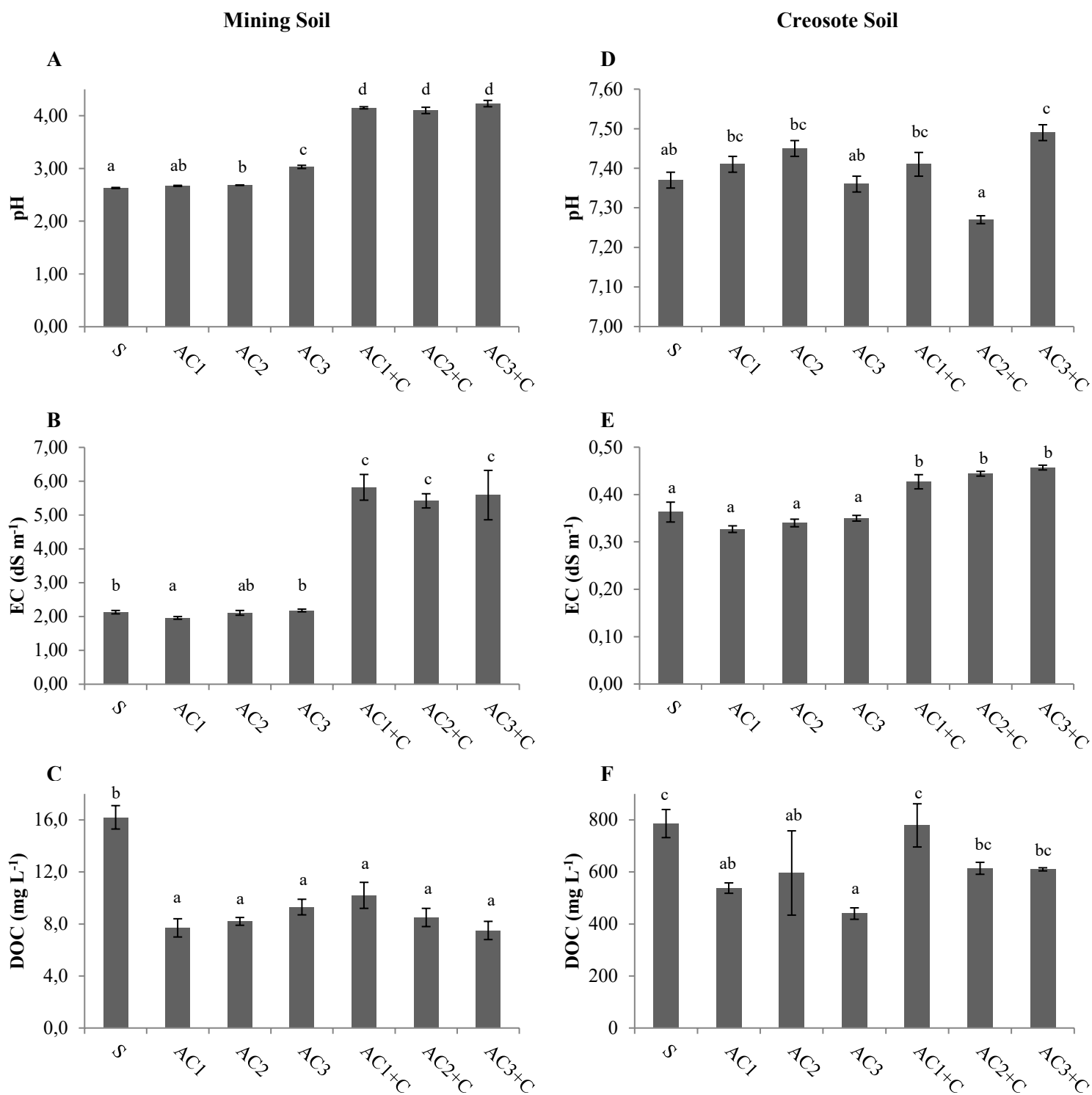


Figure 1: pH, electrical conductivity (EC) and dissolved organic carbon (DOC) measured in pore water in the mining soil (A, B and C) and in 1:10 soil:water extracts in the creosote soil (D, E and F) after 35 days of incubation. Data are mean  $\pm$  standard deviation, different letters indicate significant differences between treatments ( $n = 4$ ;  $P < 0.05$ ).

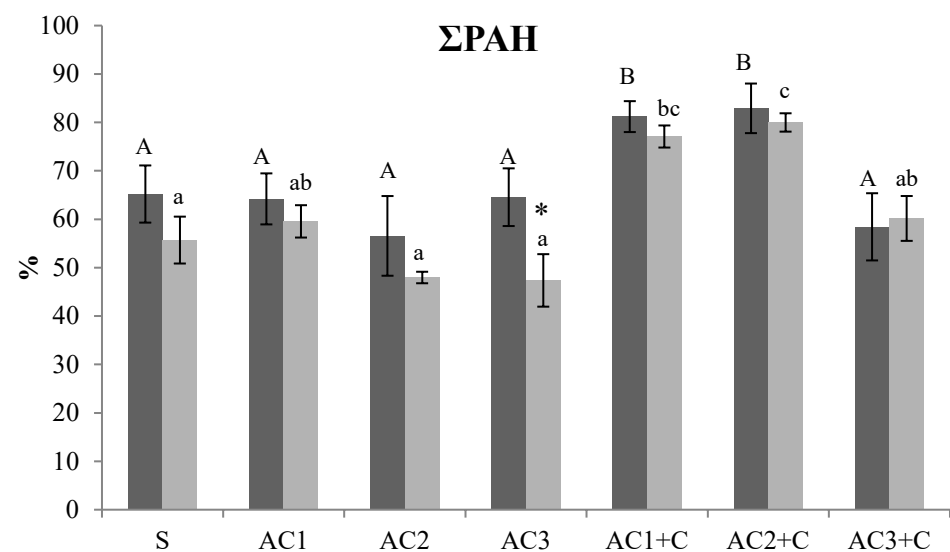
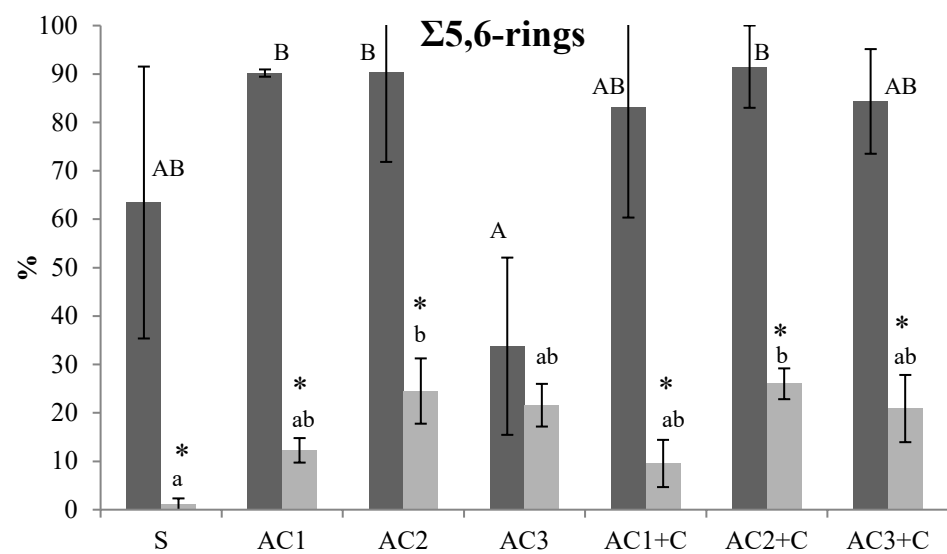
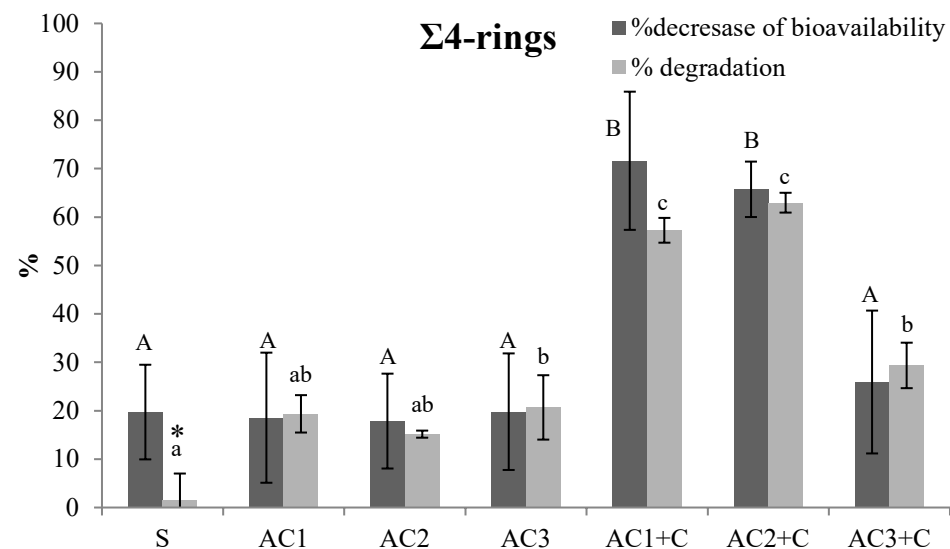
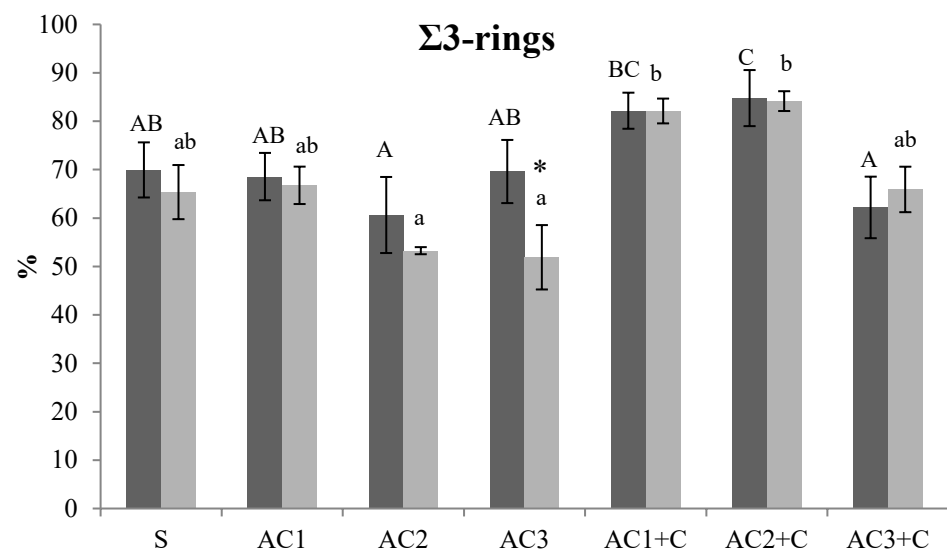


Figure 2: Percentage of decrease of bioavailability and percentage of degradation of  $\Sigma 3$ -rings,  $\Sigma 4$ -rings,  $\Sigma 5,6$ -rings and  $\Sigma$ PAH after 35 days of incubation for each microcosms (S: control soil; AC: activated carbon (1%); AC+C: activated carbon (1%) + compost (2%)) respect to initial creosote contaminated soil. Data are mean  $\pm$  standard deviation, different letters indicate significant differences between microcosms, asterisks denote significant differences between decrease of bioavailability and degradation percentage for each treatment ( $n = 4$ ;  $P < 0.05$ ).